

(a) isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA encoding a light or heavy chain of the antigen-specific antibodies and assembling the antibodies cDNA encoding said light and heavy chains of said antibodies into two separate expression cassettes, one encoding DNA for the light chain and the second encoding DNA for the heavy chain, each cassette further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus;

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(b) preparing a recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector pPICZ α by restriction digestion with EcoRI and BamHI;

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(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of step (a);

(d) cloning the expression cassettes of step (c) into the *P. pastoris* expression vector pPICZ α to generate recombinant plasmid pPICZ α LH comprising expression cassettes for the light and heavy chains;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the *Saccharomyces cerevisiae* α -mating factor signal;

(f) amplifying and isolating the recombinant plasmid;

(g) transforming *P. pastoris* spheroblasts with BglII

linearized, NotI linearized, SacI linearized, SalI linearized or Stul-linearized recombinant plasmid replacing the yeast chromosomal AOX1 DNA sequence with AOX1-antibody DNA sequence containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert;

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot;

(o) testing the recombinant antibody for specific antigen-antibody binding, and

(p) harvesting the antigen-specific antibody produced in steps (a) - (o);

wherein said antibody is produced in quantity of 10-36 mg/l in about 12 to about 108 hours.

5. (Twice Amended) The method of claim 4 wherein the antibody cDNA encoding the light and heavy chain is isolated from a hybridoma DD1 (ATCC Accession Number HB9741) that recognizes

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dioxin.
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C3
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11. (Twice Amended) The method of claim 9 wherein the screening of transformed colonies for antibody expression is by colony-immunoblotting.

19. (Twice Amended) A recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector containing dual expression cassettes, each cassette carrying an entire cDNA copy of immunoglobulin light or heavy chain DNA and further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5'-terminus and by the yeast termination DNA sequence of the 3'-terminus, said vector useful in a method for production of a recombinant antigen-specific antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

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20. (Twice Amended) An expression vector comprising *Pichia pastoris* (*P. pastoris*) transformed with human, mouse or humanized mouse immunoglobulin monoclonal cDNA for production of an entire recombinant antigen-specific intact antibody in amounts from about 10 to about 36 mg/l in about 12 to about 108 hours.

21. (Twice Amended) *Pichia pastoris* (*P. pastoris*) yeast transformed with two expression cassettes one of which carries a cDNA of a light chain of an anti-dioxin immunoglobulin and the second of which carries a cDNA of a heavy chain of an anti-dioxin